Cell Stem Cell
Previews

# Modified Patient Stem Cells as Prelude to Autologous Treatment of Muscular Dystrophy

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DOI 10.1016/j.stem.2007.11.003

**Duchenne muscular dystrophy is a devastating muscle wasting disease for which there is no effective treatment. In this issue of *Cell Stem Cell*, Benchaouir et al. (2007) demonstrate the delivery of genetically corrected CD133+ patient cells into mice, suggesting a new potential avenue for autologous cell therapy.**

Duchenne muscular dystrophy (DMD) is a progressive muscle wasting disease resulting from the absence of the 420 kDa cytoskelelal dystrophin protein. Patients are wheelchair bound by their early teens, develop heart problems, and may live into their twenties with appropriate respiratory support. Various strategies to replace the defective dystrophin protein are being tested (Muntoni and Wells, 2007; Cossu and Sampaolesi, 2007). One approach is transplantation of muscle precursor cells (myoblasts) that can produce the dystrophin protein. Myoblasts normally arise from satellite cells (on the surface of mature myofibers) that are closely associated with the microvasculature (Christov et al., 2007). The extent to which various stem cells can contribute to satellite cell and myoblast populations is a topic of intense interest. Clinical trials revealed that transplanted human myoblasts were lost rapidly and so were disappointing (Boldrin and Morgan, 2007). Alternative stem cells with myogenic potential ideally need to be derived from the patient's own cells to avoid immunorejection, and this might be possible using autologous stem cell populations isolated from a variety of adult tissues (Boldrin and Morgan, 2007; Peault et al., 2007). In this issue of *Cell Stem Cell*, Benchaouir et al. (2007) utilize the *mdx* mouse model of DMD to provide a proof of principle that human progenitor cells isolated from DMD blood and muscle biopsies can be genetically altered and then transplanted to correct the genetic deficiency of dystrophic muscle.

Circulating human CD133+ (also known as AC133+) cells exhibit limited in vitro proliferation and require coculture for myogenic fusion, but in vivo have been shown to contribute to the repair of both dystrophic muscles and endothelial cells after intra-arterial or intramuscular injection into immunocompromised *scid/mdx* mice (Torrente et al., 2004). In contrast, the present study observes excellent in vitro proliferative and inherent myogenic capacity of normal and DMD muscle-derived human CD133+ cells obtained by cell sorting from enzymatically dissociated muscle biopsies. The authors genetically transformed both blood- and muscle-derived CD133+ cells from DMD boys for use in key in vivo experiments. The isolated human cells were transduced with a lentiviral vector containing the U7 RNA to induce exon 51 skipping. Skipping this exon places the mRNA back in frame so that a truncated, but functional, dystrophin protein missing only exon 51 is transcribed (Yokata et al., 2007). The exon skipping therapeutic approach is applicable to gene defects of up to 70% of DMD patients, and avoids the problems associated with delivery of the prohibitively large full-length dystrophin gene or a (less functional) truncated minigene.

Intensive research is currently focused on delivering antisense oligonucleotides directly into DMD boys to induce skipping of the endogenous exon 51, thereby restoring expression of functional dystrophin. Indeed, two clinical trials involving intramuscular injections are already in progress (Yokata et al., 2007). Ex vivo viral transduction of autologous CD133+ cells is an alternative strategy to yield functional (exon-skipped) dystrophin+ cells for implantation into the original DMD patient. This approach avoids the major issue of immunological rejection that would occur following transplantation of heterologous normal donor (dystrophin+) myogenic cells. In the study by Benchaouir et al., it is not clear for how long the different CD133+ DMD cells were cultured prior to transduction with the U7 exon 51 lentivirus vector. The transduced human cells were transplanted after 24 hr into the right tibialis anterior muscle or the femoral artery of *scid/mdx* mice. These dystrophic scid host mice, which accept xenografts, were subjected to exercise 1 day prior to transplantation to increase muscle damage. By 3 weeks, the implanted cells gave rise to myofibers expressing the truncated human dystrophin and improved muscle function. This experiment is considered a prelude for transplantation of such genetically corrected autologous CD133+ cells back into a DMD patient.

The genetic modification of adult stem cell populations harvested from patients to produce dystrophin is not new. Cossu and colleagues have shown that modified mesoangioblasts derived from the walls of blood vessels (of mice and humans) produce dystrophin-positive myofibers in the *scid/mdx* mouse and in the dog model of DMD (Cossu and Sampaolesi, 2007). The relationship between mesangioblasts (possibly equivalent to pericytes) and

CD133+ cells such as those used in the present study is not clear. CD133+ cells are considered to be hematopoietic/endothelial stem cells of bone marrow origin that can give rise to both endothelial cells and myoblasts (Peault et al., 2007). Both mesangioblasts and CD133+ cells manifest a strong myogenic potential in vivo that can be enhanced by injury. These populations also share the advantage of being delivered via the bloodstream, and are more effective than stem cells extracted from bone marrow (Peault et al., 2007). Relevant to the crucial aspect of systemic delivery of such myogenic stem cells is the observation that intra-arterial delivery of lentivirus-transduced skeletal muscle side-population (SP) stem cells increases their myogenic contribution compared with intravenous injection, raising concerns about the likely efficacy of total systemic distribution to all muscles (Bachrach et al., 2006).

There is little doubt that it is possible to isolate populations of stem cells with the potential to repair damaged muscle and replenish the satellite cell pool, although the extent to which this occurs is debated. In a recent double-blind phase I clinical trial, autologous CD133+ cells extracted from (1 g) muscle biopsies were transplanted by intramuscular injection into eight boys with DMD and sampled after 7 months (Torrente et al., 2007). These cells were not genetically corrected, their fate was not monitored, and the boys were not immunosuppressed, because the trial was designed only to test the safety of these implanted cells (grown for only 48 hr in culture). No adverse effects were reported, although the extent to which these cells survived is not known. Increasing the efficiency of delivery of these cells, via the blood if possible, and targeting cardiac muscle remain major challenges.

The proof of principle experiments described by Benchaouir et al. in this issue show that steady progress is being made toward the goal of stem cell-mediated restoration of dystrophin expression. This approach involves substantial tissue culture and so may result in high costs that limit the number of patients who can be treated. Beyond possible safety aspects of stem cell therapy (Boldrin and Morgan, 2007), various issues remain to be clarified, including the following: the potential to enhance proliferation of blood-derived CD133+ cells in culture (and storage for repeated treatments); the time required in culture for autologous cells prior to implantation back into the patient; the patient age and associated muscle environment that the transplanted cells will encounter; the relative efficiency of blood- compared with muscle-derived cells to contribute muscle nuclei; the best route of delivery; and the longevity of the transplanted muscle nuclei in vivo (with no immunosuppression), in particular the extent to which they engraft the satellite cell compartment and thus provide a renewable source of genetically corrected stem cells within muscle. The anticipated requirement for repeated treatment cycles using this method underscores the value of blood-derived CD133+ autologous cells, compared with the need for frequent biopsies of the dystrophic muscles, and is of central importance when considering such a clinical therapy. The promising developments represented by these new sources of systemically delivered myogenic cells maintain the hope for DMD patients and their families for future autologous stem cell therapies.

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